123 Intestinal Current Measurement (ICM) in Europe: towards a harmonised protocol for clinical trials in cystic fibrosis

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Intestinal current measurement (ICM) in Ussing chambers is used to study the CFTR basic defect ex vivo in human rectal biopsies. Over the past 15 years comprehensive experience with different chamber setups and protocols (Veeze 1994, de Jonge 2004, Mall 2004) has been made in a number of European centers. Aim of this ECFS Diagnostic Network Working Group survey was to describe updated differences in methodology and a comparison of results.

All participating European centers were asked to provide details about methodology, evaluation protocol and local reference data by a standardised survey form. Original tracings and interpretation of 5 F508del homozygous CF patients and 5 healthy controls were collected from each center and centrally compared.

We confirmed differences in the European ICM practice including two types of Ussing chamber, the evaluation protocol, buffers, biopsy technique and readout parameter (Isc, Vte). Qualitative differences in CFTR chloride secretion between CF and non-CF were detected by all centers, whereas quantitative parameter mainly differed between Isc and Vte measurements and different protocol sequences.

This first European ICM survey provides a platform for the development of a harmonised ICM protocol (European SOP). Refinements in methodology and more standardised measurements according to a common protocol could help to promote the harmonisation process, and to further develop ICM as an important outcome parameter in a multicenter network for (pre-)clinical trials aiming to correct the CFTR basic defect.

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124 Target Inhalation Mode, a new Adaptive Aerosol Delivery mode for the reduction of treatment time when using the I-neb AAD System

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The I-neb AAD System (I-neb device) can be operated in Tidal Breathing Mode (TBM) or a new Target Inhalation Mode (TIM). With TBM aerosol is delivered into the first portion of each breath during tidal breathing. With TIM the patient is guided to breathe in a slow, deep inhalation, which can result in shorter treatment times.

Fifty patients with cystic fibrosis, FVC > 1 L using the I-neb device with colistimethate sodium (CMS) were included in a 3-month patient handling evaluation of the I-neb device operated in TBM and TIM. Patients were encouraged to operate the I-neb device in TIM for the delivery of all their inhaled medication. Tobramycin and hypertonic saline were delivered using two treatments with 0.5 mL metering medication chambers (MMC). Other drugs (CSM, salbutamol and dornase alfa) were delivered using a 0.3 mL MMC.

There were 10,234 complete treatments administered using TIM, and 1,675 administered using TBM. The mean treatment time in TIM was 4.27 min, compared with 6.47 min in TBM. Mean treatment times for patients using TIM were divided according to the duration of the last inhalation recorded.

Use of TIM can shorten treatment times compared with TBM, and longer last inhalation duration equated to shorter treatment times.

Table 1. Mean treatment time per last inhalation duration

Last inhalation duration recorded (s)	% of TIM patients	Mean treatment time (min)	% reduction in treatment time compared with TBM
⩽2	100	4.27	34
>2	80	4.21	35
>3	68	3.97	38
>5	48	3.70	43
=8	18	3.50	46

125 Nuclear delivery of DNA/histidine-polymer complexes: confocal microscopy real time investigation of the nuclear import of a plasmid DNA containing an optimized DNA κB sequence

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The transfection of cells by pDNA/cationic polymer complexes supposes that the delivery of pDNA in the nucleus requires polyplex capture, internalization, pDNA endosome escape and pDNA nuclear import. We have designed a new histidine-rich polyethylenimine that allows very efficient transfection with low cytotoxicity. The polymer possess protonable amines and imidazole groups in an acidic medium that favour pDNA endosome escape that favour pDNA endosome escape. We have designed an extended NFκB DNA binding sequence (so called 3NF) that is strongly recognized by NFκB upon transfection. Here, we present the quantification of the pDNA copies number in the nucleus of cells transfected with lPEI and histidine-rich PEI. We found that ~1500 copies of p3NF-luc3NF versus 250 copies of 3NF-free pDNA were imported in the nucleus upon 5 h transfection. The quantity of p3NF-luc-3NF dropped dramatically in the presence of the BAY 11–7085, an inhibitor of NFκB activation. These data strongly support the remarkable nuclear import of p3NF-luc-3NF mediated by NFκB. FRET experiments revealed that most of nuclear pDNA was still condensed with the polymer. Using cells expressing a GFP-tagged protein of the nuclear pore complex, we visualized for the first time in live cells the passage of pDNA through the nuclear pore.

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126 Optimizing aerosol administration in Cystic Fibrosis and Asthma treatment with improved Smart Card technology

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Aerosol systems used for inhalation therapy should be able to deliver medication to the region of the respiratory system where the drug is required to optimize efficacy. However, efficiency and reproducibility of particle deposition in the lung in most of the commercially available aerosol systems is limited by the breathing pattern of the patients.

The AKITA JET inhalation system was developed to guide the patient through the inhalation using a controlled flow rate and inhalation volume. In order to both individualize breathing pattern as well as record compliance data of therapy, an improved Smart Card technology is incorporated into the system. In most countries, two 'standard' Smart Cards are enclosed to the system containing the following medications for treatment of cystic fibrosis and severe asthma diseases:

Cystic Fibrosis:
- Atrovent/Sultanol
- Tobramycine
- DNAse
- Colistin

Asthma and COPD:
- Atrovent/Sultanol
- Fluticasone
- Budesonide

The Smart Cards are programmed in a way to count down the number of breaths. A pre-selectable inspiration time per breath and therefore a certain number of breaths for every medication assure correct dosage and reproducible drug delivery for every treatment.

Aerosol characterizations showed (for all medications) a particle size (MMD by laser diffraction) of 3.76 μm (GSD 1.99) and delivered dose of 41–57% of filling dose independent from the pre-selected inspiration time per breath.

Controlled breathing reduces inhalation treatment time since lung deposition of inspired particles is higher if compared to conventional devices where patients are free to inhale spontaneously. AKITA JET is a highly efficient nebulizer system that ensures high and homogeneous lung deposition of different drugs.